results suggest that post-transplant IL-22 administration represents a novel strategy to reduce gut GVHD by direct protection of intestinal epithelium without limiting immune function post-transplant.

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Targeting Sag in Donor T Cells As a Novel Strategy for Reducing Gvhd

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Neddylation is crucial for the degradation of certain proteins. However its role in regulating T cells is unknown. Neddylation is mediated by cullin-RING ligase (CRL) protein complex, an E3 ubiquitin ligase and its critical adapter element, SAG protein (sensitive to apoptosis gene protein). We explored the role of SAG and thus neddylation in T cells by utilizing two different, but complementary approaches, namely, genetic knock-out and chemical inhibition with small molecule. The T cell specific SAG KO animals were generated by crossing B6 $SAG^{fl/fl}$ mice with B6 LCK-Cre mice. The KO animals were viable. The splenic and thymic analyses showed no significant differences in the numbers of conventional T cells (Tcons) and Tregs between the KO and WT animals. In vitro functional analysis of Tcons, however, revealed that stimulation with either allogeneic splenocytes or by α-CD3 and α-CD28 antibody, $SAG^{-/-}$ T cells showed significantly decreased proliferation ($P<0.002$). Phenotypic analysis following stimulation demonstrated that $SAG^{-/-}$ T cells showed reduced expression of CD69, CD44 and greater expression of CD62L when compared to WT-T cells ($P<0.04$). The KO-T cells also demonstrated reduced expression of T effector signature cytokines, IL-17, IFN-γ and IL-4. Similar reduction in proliferation, activation marker expression and release of cytokines was observed when the WT-T cells were treated with small molecule inhibitor of neddylation, MLN4924.

We next determined the in vivo relevance of SAG and neddylation in Tcons by utilizing the MHC disparate (B6→BALB/c) model of allogeneic BMT. The BALB/c animals were lethally irradiated and transplanted with TCD BM from either syngeneic or allogeneic WT-B6 animals along with $5x10^5$ splenic T cells from either the WT B6 or $SAG^{-/-}$ B6 animals. The allogeneic animals that received $SAG^{-/-}$ T cells demonstrated markedly reduced clinical GVHD and significantly increased survival when compared to those that received WT-B6 T cells ($P<0.001$). Similar results were observed in B6→B6D2F1 model. To further confirm our results and to determine potential translational application, we utilized the small molecule MLN4924, once again in the B6→BALB/c system. The recipient mice were lethally irradiated and received 5 doses of MLN4924 (20mg/kg, day-1 to day +3 of BMT) along with WT-B6 T cells. Mice receiving MLN4924 demonstrated significantly decreased clinical GVHD and improved survival. Our studies thus demonstrate that SAG is a novel molecular target for regulating T cell responses and mitigating GVHD. Furthermore, the clinical availability of the small molecule, MLN4924, suggests that this strategy could be tested in carefully designed human clinical trial for attenuating GVHD.

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Sensitization to HY-Antigen in Female Donors Was Not Associated with the Post-Transplant HY-IgG Development Nor Clinical Outcomes in Sex-Mismatched Transplantation

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Background: Transplant of male recipients from female donors (F>M HCT) is well known as a risk factor for developing chronic graft-versus-host disease (cGVHD). We have so far suggested that B cell response against minor histocompatibility antigens encoded on the Y chromosome, called H-Y antigens, develops following F>M HCT and associates with cGVHD. Here, we hypothesize that pre-sensitization to HY-antigen in a female donor may affect the post-HCT HY-IgG development and clinical outcomes following F>M HCT. This study uses our novel HY microarray to determine the prevalence and impact of donor HY-IgG.

Methods: We measured IgG against 5 HY antigens (DBY, UTY, ZFY, EIF1AY, & RBS4Y) in 289 female donors (age:18-60) of high resolution 8/8 HLA-matched HCT facilitated by the NMDP between 1990-2002 and assessed the impact of HY seropositivity on cumulative cGVHD incidence and other clinical outcomes.

In addition, we studied 90 Stanford adult female donors and their corresponding male recipients between 2005 and 2012 who survived without relapse for at least 3m post-HCT and assessed the association of HY-IgG development between pre- and post-HCT. The cut-off value for seropositivity was defined as Q3 + 2xIQR, determined from plasma of 60 maledonors. HY-score was defined as the cumulative number of targeted HY antigens.

Results: Prevalence of HY-IgGs in female donors is shown in Table1. Half of female donors had at least one of 5 HY-IgG(s). Univariate analyses of NMDP cohort showed that individual HY-IgGs in female donors were not associated with cGVHD. Focusing on increasing HY-score, we did not detect association with cGVHD nor other clinical outcomes (Table 2). This absence of association was also observed in Stanford cohort. Further, we were unable to show the

Table 1

	DBY	UTY	ZFY	EIF1AY	RPS4Y	Any-HY
NMDP (n=289)	63 (22%)	112 (39%)	22 (8%)	7 (2%)	42 (15%)	143 (49%)
Stanford (n=90)	20 (22%)	32 (36%)	4 (4%)	1 (1%)	8 (9%)	46 (51%)

Table 2

(NMDP)	cGVHD		aGVHD		Relapse		TRM		OS	
HY-score	HR	P	HR	P	HR	P	HR	P	HR	P
0 (n=146)	1	-	1	-	1		1	-	1	-
1 (n=75)	1.34	0.15	1.17	0.46	1.22	0.54	0.85	0.44	0.98	0.89
2 (n=39)	1.12	0.65	1.25	0.39	0.63	0.31	0.62	0.078	0.81	0.39
3 to 4 (n=29)	1.32	0.37	1.64	0.064	1.85	0.19	1.3	0.34	1.48	0.099